

US006225445B1

(12) United States Patent

Shen et al.

(10) Patent No.: US 6,225,445 B1

(45) **Date of Patent:** *May 1, 2001

(54) METHODS AND COMPOSITIONS FOR LIPIDIZATION OF HYDROPHILIC MOLECULES

(75) Inventors: Wei-Chiang Shen, San Marino;

Hossein M. Ekrami, Los Angeles, both

of CA (US)

(73) Assignee: The University of Southern

California, Los Angeles, CA (US)

(*) Notice: This patent issued on a continued prosecution application filed under 37 CFR

1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C.

154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35

U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/120,118

(22) Filed: Jul. 22, 1998

Related U.S. Application Data

- (60) Division of application No. 08/524,362, filed on Sep. 5, 1995, now Pat. No. 5,907,030, which is a continuation-in-part of application No. 08/349,717, filed on Jan. 25, 1995, now abandoned.
- (51) **Int. Cl.**⁷ **C07K 1/00**; C07K 14/00; C07K 16/00; A61K 35/14; A61K 35/16
- (52) **U.S. Cl.** **530/350**; 530/380; 530/385; 530/387.1

(56) References Cited

U.S. PATENT DOCUMENTS

5,599,903	2/1997	Kauvar et al 530/331
5,629,020	5/1997	Leone-Bay et al 424/489
5,635,380	6/1997	Naftilan et al 435/172.3
5,679,643	10/1997	Kauvar et al 514/18
5,763,570	6/1998	Kauvar et al 530/331

FOREIGN PATENT DOCUMENTS

0 482 766 4/1992 (EP) . WO 91/16067 10/1991 (WO) .

OTHER PUBLICATIONS

Webster's Third New International Dictionary, Unabridged, G & C.Merriam Co.., Publishers, 1963.*

Artursson, P. and C. Magnusson, "Epithelial Transport of Drugs in Cell Culture. II: Effect of Extracellular Calcium Concentration on the Paracellular Transport of Drugs of Different Lipophilicities across Monolayers of Intestinal Epithelial (Caco-2) Cells," *J. Pharm. Sci.* 79(7):595–600 (1990).

Broadwell, R. et al., "Transcytotic pathway for blood-borne protein through the blood-brain barrier," *Proc. Natl. Acad. Sci. USA* 82(2):632–636 (1988).

Chekhonin, V. et al., "Fatty acid acylated Fab-fragments of antibodies to neurospecific proteins as carriers for neuroleptic targeted delivery in brain," *FEBS Letters* 287(1,2):149–152 (1991).

Chu, Y.-C. et al., "High-Potency Hybrid Compounds Related to Insulin and Amphioxus Insulin-like Peptide," *Biochem.* 33(44):13087–13092 (Nov. 1994).

Chu, Y.-C. et al., "High-Potency Hybrid Compounds Related to Insulin and Amphioxus Insulin-like Peptide," *Chem. Abstracts* 121(21):212, Abstract No. 246582y (Nov. 1994).

Conradi, R. et al., "The Influence of Peptide Structure on Transport Across Caco-2 Cells," *Pharm. Res.* 8(12):1453–1460 (1991).

Edwards, P., "Is Mucus A Selective Barrier To Macromolecules?" *British Med. Bulletin 34(1)*:55–56 (1978).

Ekrami, H., "Positively-Charged and Lipophilic Bowman-Birk Protease Inhibitor Conjugates: Synthesis and Characterization of the Pharmaceutical and Chemopreventive Properties," A Dissertation, UMI Dissertation Services, Ann Arbor, MI, Chapter II, pp. 98–226 (after Jan. 25, 1995). Ekrami, H. et al., "Water-soluble fatty acid derivatives as acylating agents for reversible lipidization of polypeptides," *FEBS Letters* 371(3):283–286 (Sep. 1995).

Ekrami, H. et al., "Water-soluble fatty acid derivatives as acylating agents for reversible lipidization of polypeptides," *Chem. Abstracts* 124(3):1042, Abstract No. 30361q (Jan. 1996).

Fix, J. et al., "Acylcarnitines: drug absorption-enhancing agents in the gastrointestinal tract," *Amer. J. Physiology* 251(3, Prt 1):G332-G340 (1986).

Gonzalez-Mariscal, L. et al., "Tight Junction Formation in Cultured Epithelial Cells (MDCK)," *J. Membrane Biol.* 86(2):113–125 (1985).

Gordon, G. et al., "Nasal absorption of insulin: Enhancement by hydrophobic bile salts," *Proc. Natl. Acad. Sci. USA* 82(21):7419–7423 (1985).

Hashimoto, M. et al., "Synthesis of Palmitoyl Derivatives of Insulin and Their Biological Activites," *Pharm. Res.* 6(2):171–176 (1989).

Huang, W. et al., "Lipophilic Multiple Antigen Peptide System For Peptide Immunogen And Synthetic Vaccine," *Mol. Immunol.* 31(15):1191–1199 (Oct. 1994).

(List continued on next page.)

Primary Examiner—Alan L. Rotman (74) Attorney, Agent, or Firm—Sterne, Kessler, Goldstein & Fox, P.L.L.C.

(57) ABSTRACT

Fatty acid derivatives of sulfhydryl-containing compounds (for example, sulfhydryl-containing peptides or proteins) comprising fatty acid-conjugated products with a disulfide linkage are employed for delivery of the compounds to mammalian cells. This modification markedly increases the absorption of the compounds by mammalian cells relative to the rate of absorption of the unconjugated compounds, as well as prolonging blood and tissue retention of the compounds. Moreover, the disulfide linkage in the conjugate is quite labile in the cells and thus facilitates intracellular release of the intact compounds from the fatty acid moieties.

17 Claims, 8 Drawing Sheets-